



***Antonbruunia sociabilis* sp. nov. (Annelida: Antonbruunidae) associated with the chemosynthetic deep-sea bivalve *Thyasira scotiae* Oliver & Drewery, 2014, and a re-examination of the systematic affinities of Antonbruunidae**

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Abstract

Antonbruunia sociabilis sp. nov., an abundant endosymbiont of *Thyasira scotiae* from a putative sulphidic ‘seep’ in the Hatton-Rockall Basin (1187–1200 m), North-East Atlantic Ocean, is described. The new species is compared with *A. viridis* and *A. gerdesi* from the West Indian Ocean and South-East Pacific Ocean respectively. The three species can be distinguished using a suite of morphological characters, and are associated with geographically separated chemosynthetic bivalve molluscs from different families (Thyasiridae, Lucinidae, Vesicomidae) living in sediments at different depths. New morphological features are recognized for *Antonbruunia* and a re-assessment of its systematic affinities indicates a close relationship with the Pilargidae. Previous suggestions of an affiliation with the Nautiliniellidae, recently incorporated into the Calamyzinae (Chrysopetalidae), were not supported. The apparent morphological similarities between the two groups are indicative of convergence related to their shared relationships with chemosynthetic bivalves. The first molecular analyses of *Antonbruunia* (16S and 18S rDNA) clearly indicate that a close relationship to Pilargidae (represented by *Ancistrostylis* sp. and *Sigambra* sp.) is more likely than an affinity to Calamyzinae (represented by *Calamyzas amphictenicola*, *Natushima* sp., and *Vigtorniella* sp.).

Key words: Polychaete, mollusc, symbiosis, NE Atlantic, morphology, molecular analyses, relationships

Introduction

Antonbruunidae Fauchald, 1977, comprises a single genus *Antonbruunia* Hartman & Boss, 1965 and two species: *A. viridis* Hartman & Boss, 1965 and *A. gerdesi* Quiroga & Sellanes, 2009. Both species are associated with chemosynthetic bivalve molluscs, living freely within the mantle cavities of *Lucina fosteri* Hartman & Boss, 1965 (Lucinidae J. Fleming) and *Calypptogena gallardoi* Sellanes & Krylova, 2005 (Vesicomidae Dall & Simpson), respectively. *Antonbruunia viridis* and its host bivalve are only known from shallow water (68–82 m) sediments “of black-brown oozy mud and detritus” off southwest Madagascar, Western Indian Ocean, while *A. gerdesi* and its host occur in the “sticky dark grey sediments smelling of sulphide at a deeper water (795–846 m) cold seep location off central Chile, South-East Pacific Ocean.

In June 2012, two unusual bivalves were collected from 1187–1200 m in the Hatton-Rockall Basin (North-East Atlantic Ocean), 500 km west of the Isle of Harris, Outer Hebrides, Scotland. These new species were described as *Isorropodon mackayi* Oliver & Drewery, 2014 and *Thyasira scotiae* Oliver & Drewery, 2014 and, from their chemosymbiotic nature, it was deduced that they inhabited sulphidic sediments associated with an active cold seep. The latter species, on dissection, frequently had numerous individuals of a new species of *Antonbruunia* within. The species is described herein and its morphology compared with *A. viridis* and *A. gerdesi*.

The systematic affinities of *Antonbruunia* have always been unclear. Hartman & Boss (1965) considered it to have some similarities with the Pilargidae de Saint-Joseph, but possessing modifications for a commensal existence. Salazar-Vallejo (1986) re-assessed the characteristics of the genus and regarded the establishment of

Antonbruunidae unwarranted; *Antonbruunia* and seven other genera were placed in Sigambrinae Salazar-Vallejo (= Pilarginae, see Salazar-Vallejo & Orensanz 1991). Soon after, a new genus and species of polychaete, *Nautilina calyptogenicola* Miura & Laubier, 1989, was described from the mantle cavity of the giant deep-sea clam *Calyptogena phaseoliformis* Métivier, Okutani & Ohta, 1986 inhabiting a cold seep zone in the Japan Trench (5960 m). A new family, Nautilinidae Miura & Laubier, 1989, was erected and the authors discussed its similarities with Calamyzidae Hartmann-Schröder and Levidoridae Perkins. *Nautilina* was preoccupied and subsequently replaced by *Nautiliniella* Miura & Laubier, 1990, and the family corrected to Nautiliniellidae Miura & Laubier.

In addition, the authors acknowledged resemblances with Antonbruunidae and re-examined paratype material of *A. viridis*. They concluded that the two families were morphologically distinct and that the Nautiliniellidae was “near the family Pilargidae.”

Glasby (1993) carried out a cladistic analysis of the superfamily Nereidoidea *sensu* George & Hartmann-Schröder, 1985 and also re-examined *A. viridis*. However, he synonymised Antonbruunidae with Pilargidae. Calamyzidae and Levidoridae were synonymised with Syllidae Grube while the Nautiliniellidae were redefined. Pilargidae, Nautiliniellidae and Syllidae formed a monophyletic group, sister to the Nereididae Blainville, Chrysopetalidae Ehlers and Hesionidae Grube. In the analyses of Pleijel & Dahlgren (1998) the position of *Antonbruunia* was variable and they concluded that the relationships between Antonbruunidae, Nautiliniellidae and Pilargidae merited further investigation. Martin & Britayev (1998) reviewed symbiotic polychaetes and speculated that further investigation would probably result in Nautiliniellidae becoming a synonym of Antonbruunidae, although later in the same paper they referred to *Antonbruunia viridis* as a pilargid! Large-scale works on polychaetes (Beesley *et al.* 2000; Rouse & Pleijel 2001) reiterated the uncertainties surrounding the status of Antonbruunidae.

Recently, Aguado *et al.* (2013) carried out a morphological and molecular study on the affinities of the Nautiliniellidae. They found a Calamyzinae clade (= Nautiliniellidae + *Calamyzas* + chrysopetalid *Vigtorniella*) nesting together with Dysponetinae Aguado, Nygren & Rouse and Chrysopetalinae Ehlers within a monophyletic Chrysopetalidae. These newfound relationships drew attention to the Antonbruunidae once more, and Aguado *et al.* noted the morphological similarities with Calamyzinae and their shared symbiosis with bivalves. In this paper we re-examine the morphological features of Antonbruunidae and, together with the first molecular analyses of a species of *Antonbruunia*, further investigate its systematic position.

Material and Methods

Thyasira scotiae specimens containing the *Antonbruunia* material were collected by a Jackson 460 Otter trawl deployed (Haul S12283a, 1187–1200 m) in the Hatton-Rockall Basin during RV *Scotia* research cruise 0712S as part of the Scottish Government funded OFFCON project. Full sampling details and location map are provided in Oliver & Drewery (2014). All bivalve material was preserved directly in 70% ethanol.

Morphological examinations. Nineteen *Antonbruunia* specimens (eight, nine, and two respectively) were removed from the three *Thyasira scotiae* type specimens (NMW-Z.2012.074.4-5) by the second author and placed in 100% ethanol. Of these, two paratypes (1, 1, -) were critically point dried, sputter coated with gold and imaged using a Jeol Neoscope JCM-5000 Scanning Electron Microscope (SEM). The holotype (-, -, 1) and remaining paratypes (5, 6, 1) were examined and measured using a Wild M8 stereo-zoom microscope. One paratype (1, -, -) was later extensively dissected, slide mounted, and additionally examined using a Nikon Labophot-2 compound microscope. Drawings were made using camera lucida attachments on both microscopes. The holotype was temporarily stained with Shirlastain A (see Petersen 1998) to aid drawing. Assessment of epidermal glands was carried out using Methyl Green staining as in Mackie & Gobin (1993). The remaining four specimens were selected (2, 2, -) for molecular analysis. Type material is deposited in the zoological collections of the National Museum Wales (NMW-Z).

Phylogenetic analyses. In addition to *Antonbruunia* we included 29 taxa from 17 families in Aciculata.

TABLE 1. Taxa, GenBank accession numbers and references for 18S rDNA and 16S rDNA sequences.

Family	Genus/Species	18S	Reference	16S	Reference
Amphinomidae	<i>Eurythoe complanata</i>	AY364851	Jördens <i>et al.</i> 2004	JN086557	Borda <i>et al.</i> 2012
	<i>Paramphinode jeffreysi</i>	AY838856	Struck <i>et al.</i> 2006	AY838840	Struck <i>et al.</i> 2006
Antonbruunidae	<i>Antonbruunia sociabilis</i>	KP992843	This paper	KP992845	This paper
Aphroditidae	<i>Aphrodita aculeata</i>	AY176281	Worsaae <i>et al.</i> 2005	JN852882	Norlinder <i>et al.</i> 2012
Chrysopetalidae	<i>Bhawania heteroseta</i>	EU555035	Wiklund <i>et al.</i> 2009	EU555044	Wiklund <i>et al.</i> 2009
	<i>Calamyzas amphictenicola</i>	JX078939	Aguado <i>et al.</i> 2013	JX093563	Aguado <i>et al.</i> 2013
	<i>Natsushima</i> sp.	JX078944	Aguado <i>et al.</i> 2013	JX078954	Aguado <i>et al.</i> 2013
	<i>Vigtorniella flokati</i>	EU555043	Wiklund <i>et al.</i> 2009	EU555034	Wiklund <i>et al.</i> 2009
Glyceridae	<i>Glycera dibranchiata</i>	AY995208	Struck <i>et al.</i> 2006	AY995209	Struck <i>et al.</i> 2006
Goniadidae	<i>Glycinde armigera/multidens</i>	DQ790079	Struck <i>et al.</i> 2007	GQ426612	Böggemann 2009
	<i>Goniada brunnea/vorax</i>	DQ790080	Struck <i>et al.</i> 2007	GQ426613	Böggemann 2009
Hesionidae	<i>Nereimyra punctata</i>	AY176294	Worsaae <i>et al.</i> 2005	DQ442577	Ruta <i>et al.</i> 2007
	<i>Oxydromus flexuosus</i>	EU555039	Wiklund <i>et al.</i> 2009	DQ442578	Ruta <i>et al.</i> 2007
Lacydoniidae	<i>Lacydonia</i> sp.	AY996082	Eklöf <i>et al.</i> 2007	AY996061	Eklöf <i>et al.</i> 2007
Lumbrineridae	<i>Ninoe nigripes</i>	AY838852	Struck <i>et al.</i> 2006	AY838837	Struck <i>et al.</i> 2006
Nephtyidae	<i>Aglaophamus malmgreni</i>	AY996091	Eklöf <i>et al.</i> 2007	AY996070	Eklöf <i>et al.</i> 2007
	<i>Nephtys incisa</i>	EU418857	Struck <i>et al.</i> 2008	GU179356	Ravara <i>et al.</i> 2010
Nereididae	<i>Ceratocephale loveni</i>	DQ442616	Rousset <i>et al.</i> 2007	DQ442614	Rousset <i>et al.</i> 2007
	<i>Nereis pelagica</i>	AY612621	Persson & Pleijel 2005	AY340470	Rousset <i>et al.</i> 2007
Oeonidae	<i>Drilonereis longa</i>	AY838847	Struck <i>et al.</i> 2006	AY838828	Struck <i>et al.</i> 2006
Paralacydoniidae	<i>Paralacydonia paradoxa</i>	DQ790088	Struck <i>et al.</i> 2007	GQ426619	Böggemann 2009
Phyllodocidae	<i>Eulalia viridis</i>	AY996085	Eklöf <i>et al.</i> 2007	AY340455	Rousset <i>et al.</i> 2007
	<i>Phyllodoce longipes</i>	AY996075	Eklöf <i>et al.</i> 2007	AY996056	Eklöf <i>et al.</i> 2007
Pilargidae	<i>Ancistrosyllis</i> sp.	AF474280	Nygren & Sundberg 2003	KP992844	This paper
	<i>Sigambra</i> sp.	AY340444	Rousset <i>et al.</i> 2007	AY340481	Rousset <i>et al.</i> 2007
Sigalionidae	<i>Sthenelais boa</i>	DQ779672	Rousset <i>et al.</i> 2007	DQ779635	Rousset <i>et al.</i> 2007
Sphaerodoridae	<i>Sphaerodoropsis anae</i>	EF123871	Aguado <i>et al.</i> 2007	EF123798	Aguado <i>et al.</i> 2007
Syllidae	<i>Eusyllis blomstrandii</i>	AF474281	Nygren & Sundberg 2003	EF123788	Aguado <i>et al.</i> 2007
	<i>Proceraea cornuta</i>	AF474312	Nygren & Sundberg 2003	AF474266	Nygren & Sundberg 2003
	<i>Syllides fulvus</i>	EF123839	Aguado <i>et al.</i> 2007	EF123804	Aguado <i>et al.</i> 2007

Data retrieval and handling. All sequences except for *Antonbruunia* and the 16S rDNA sequence of *Ancistrosyllis* were retrieved from GenBank (Table 1). DNA was extracted using DNAeasy Tissue Kit (Qiagen) following the protocol supplied by the manufacturer. We used the primers 16SarL (CGCCTGTTTATCAA AAACAT) and 16SbrH (CCGGTCTGAAGTACGATCACGT) (Palumbi 1996) for 16S rDNA, and PCR1F (AYCTGGTTGATCCTGCCAGT), PCR2F (TAAAGYTGYTGCAGTTAAA), PCR1R (TASGACGGTATCTGA TCGTCTT), and PCR2R (ACCTTGTTACGACTTTTACTTCCTC) (Nygren & Sundberg 2003) for 18S rDNA. PCR mixtures contained 21 µl ddH₂O, 1 µl of each primer (10 µM), 2 µl of DNA template, and puReTaq Ready-To-Go PCR Beads (Amersham Biosciences). The temperature profile was as follows: 96°C/240s–(94°C/30s–48–62°C/

30s–72°C/ 60s)*45 cycles –72°C/480s. PCR products were purified with the E.Z.N.A. Cycle-Pure Kit (Omega Bio-tek). Sequencing was performed at Macrogen Inc. facilities (Seoul, Korea). Overlapping sequence fragments were merged into consensus sequences using Geneious version 7.0.6 available from <http://www.geneious.com/>. We used MAFFT v7.017 (Kato *et al.* 2002) within Geneious 7.0.6 with the following settings: algorithm=E-INS-i, scoring matrix=200PAM / k=2, gap open penalty=1.53 to align the sequences. We used the online GBlocks server v. 0.91b (Castresana 2002), using the option ‘Allow gap positions within the final blocks’, to detect alignment-ambiguous sites that were subsequently excluded from the analysis (Gatesy, DeSalle & Wheeler 1993; Castresana 2000; Talavera & Castresana 2007). The gene partitions were concatenated using Mesquite v. 2.75 (Maddison & Maddison 2008).

Analytical procedures. The mitochondrial and nuclear data sets were analysed separately and combined using Bayesian inference (BA), and Maximum Likelihood (ML). Bayesian analyses (BAs) of separate and combined data sets were run in MrBayes 3.2 (Ronquist & Huelsenbeck 2003), and the best-fit models were selected using the Akaike information criterion in MrModeltest 2.1 (Nylander 2004). We used a general time reversible model (GTR), with gamma distributed rate across sites (GTR+G) for 16S rDNA, and a GTR+G with a proportion of the sites invariable (I) for 18S rDNA. The partitions were unlinked in the combined analysis. The number of generations was set to 10 million, with four parallel chains (three hot, one cold), sample frequency was set to 1000, and number of runs set to two. One third of the samples were discarded as burn-ins. Maximum likelihood analyses (MLs) were performed in raxmlGUI (Silvestro & Michalak 2012). In RAxML, the analyses were run with the GTRGAMMA model, the combined data set was partitioned as in BA, and clade support was assessed using 1000 bootstrap replicates.

Morphological Results

Antonbruunia Hartman & Boss, 1965 emended

Diagnosis. Prostomium anteriorly bearing pair of antero-ventral palps, pair of dorso-lateral antennae, and a postero-dorsal median antenna; eyes lacking. Nuchal organs pair of dorso-lateral ciliated grooves between posterior prostomium and anterior margin of segment 1. Peristomium ventrally fused with prostomium and segment 1, bearing transverse slit-like mouth. Two pairs of tentacular cirri on segment 1; parapodia and chaetae lacking. Anterior few segments with short muscular axial pharynx, proboscis short and bulbous, jaws lacking; gut tubular, lateral gut caecae may be present, extending into parapodia. Body and parapodia smooth, lacking papillae. Parapodia simple, with numerous bidentate neurochaetae in two groups, one above and one below several parallel neuroaciculae, and separate single notoacacula (not associated with dorsal cirri); lacking well-developed pre- or post-chaetal lobes or lamellae. Dorsal cirri larger than ventral cirri. Single pair of pygidial cirri. Sexes may be dimorphic with smaller males.

Remarks. Fauchald (1977) interpreted the four anterior antennae as two palps and two antennae. This has been followed by most subsequent workers (e.g. Pleijel 2001c; Aguado *et al.* 2013). The diagnosis is emended following observations herein (see below) concerning the nuchal organs, notoacacula and parapodia, and segmental gut caecae.

Antonbruunia sociabilis sp. nov.

Figs 1–4

Material examined. NE Atlantic: Scotland, Hatton-Rockall Basin, FRV *Scotia*, cruise 0712S, Station S12283a, 57°57'N 15°33'W, 1187–1200 m, Jackson 460 Otter trawl, 23 June 2012.

Antonbruunia removed from among arborescent lateral body pouches of three *Thyasira scotiae* types (NMW-Z.2012.074.4–5); eight, nine, and two specimens, last including holotype (NMW-Z.2012.074.6) and one paratype (NMW-Z.2012.074.7).

Remaining types. Four paratypes (NMW-Z.2012.074.8), one paratype (NMW-Z.2012.074.9 dissected), one paratype (NMW-Z.2012.074.10 SEM), six paratypes (NMW-Z.2012.074.11) and one paratype (NMW-

Z.2012.074.12 SEM). Four specimens were selected for sequencing; one voucher (NMW-Z.2012.074.13) yielded the sequences registered with GenBank (Table 1).

Description. Holotype entire, maximum width 1.08 mm (including parapodia), 18.5 mm long for prostomium and 48 segments. Paratypes ranging from 0.56–1.28 mm wide, 5.2–22.6 mm long, and 32–52 segments.

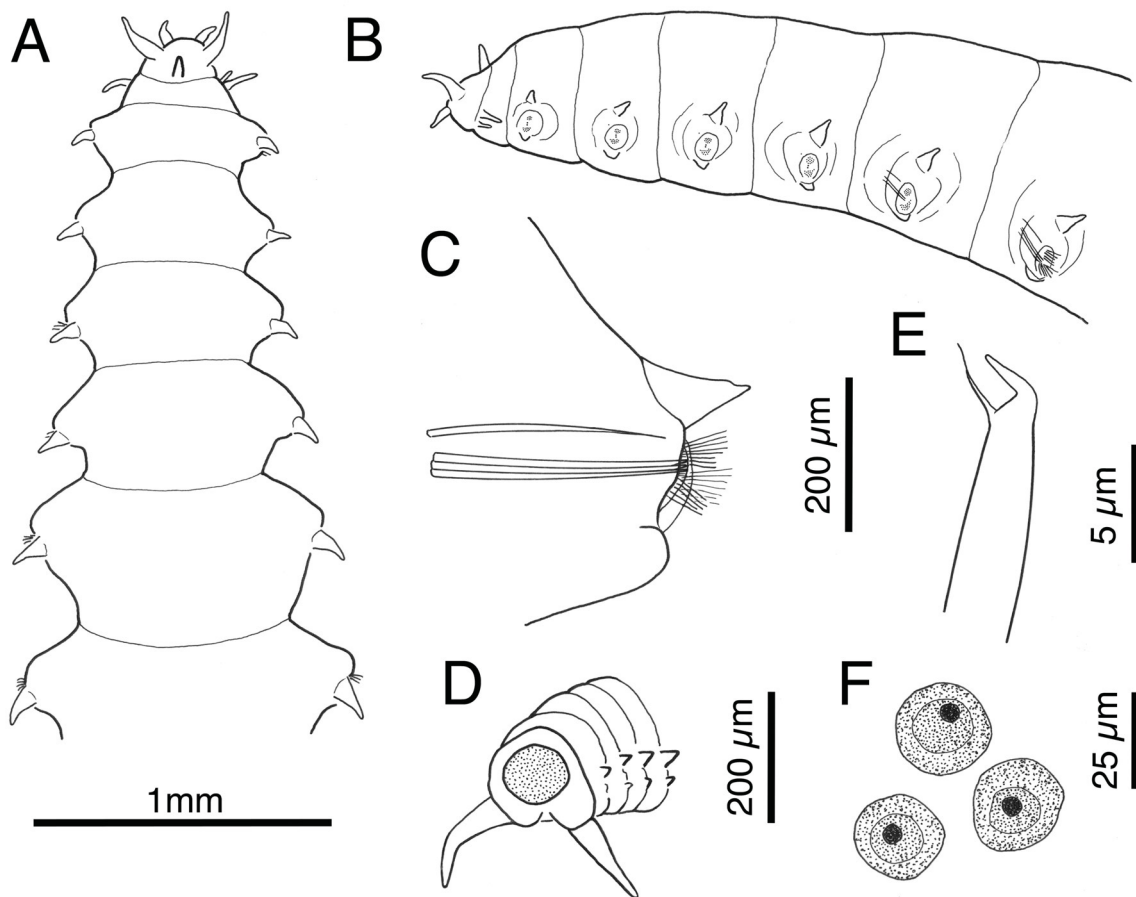


FIGURE 1. *Antonbruunia sociabilis* sp. nov. (A, B & D, Holotype NMW-Z.2012.074.6; C & E, Paratype NMW-Z.2012.074.9; F, Largest paratype NMW-Z.2012.074.8). A, anterior region, dorsal view; B, Anterior region, lateral view; C, parapod, chaetiger 16, anterior view; D, Posterior region; E, neuropodial chaeta, distal region, chaetiger 17; F, oocytes.

Body long and slender, dorsally arched and ventrally flat, lacking ventral groove. Head and anterior 5 segments narrow; segments thereafter rapidly increasing in size (Fig. 1A–B), reaching maximum in anterior third of body, rapidly decreasing in posterior region. Cuticle smooth and shiny on best preserved specimens. Pygidium lobulate, ventro-laterally with single pair of tapered pygidial cirri (Figs 1D, 2D).

Prostomium subtrapezoidal, with rounded anterior margin, lacking eyes (Fig. 1A). Pair of antero-ventral palps, somewhat conical, basally expanded and distally tapered. Pair of antero-dorsolateral antennae, of similar shape to palps, but broader and longer (Fig. 2A–B). Median antenna of similar size and shape to palps, arising mid-dorsally near posterior margin of prostomium (Fig. 1A,B). Nuchal organs elliptical ciliated grooves, positioned dorso-laterally between posterior prostomium and anterior margin of segment 1 (Fig. 2B).

Peristomium ventrally fused with prostomium and segment 1; delineation between prostomium and segment 1 only distinct dorsally and laterally. Mouth ventral, transverse, slit-like (Fig. 2A–B). Two pairs of tapered tentacular

cirri on segment 1, superior cirri 1.5 to 2 times as long as inferiors; parapodia and chaetae lacking. Pharynx muscular, axial, short and narrow, occupying anterior three segments; proboscis short and bulbous when extruded (Fig. 2C), jaws lacking. Gut tubular, with markedly wider lumen from anterior to middle of chaetiger 3. Gut caecae from chaetiger 3 as pair of small lateral pouches; caecae increase in size over following 2–3 chaetigers, extending into parapodia. By chaetiger 6 or 7, distally tapered caecae reach into posterior parts of parapodia, just below dorsal cirri; caecae decrease in size in posterior half to third of body. Gut caecae revealed through methyl green staining; caecae appearing as opaque white unstained masses either side, extending laterally into the parapodia.

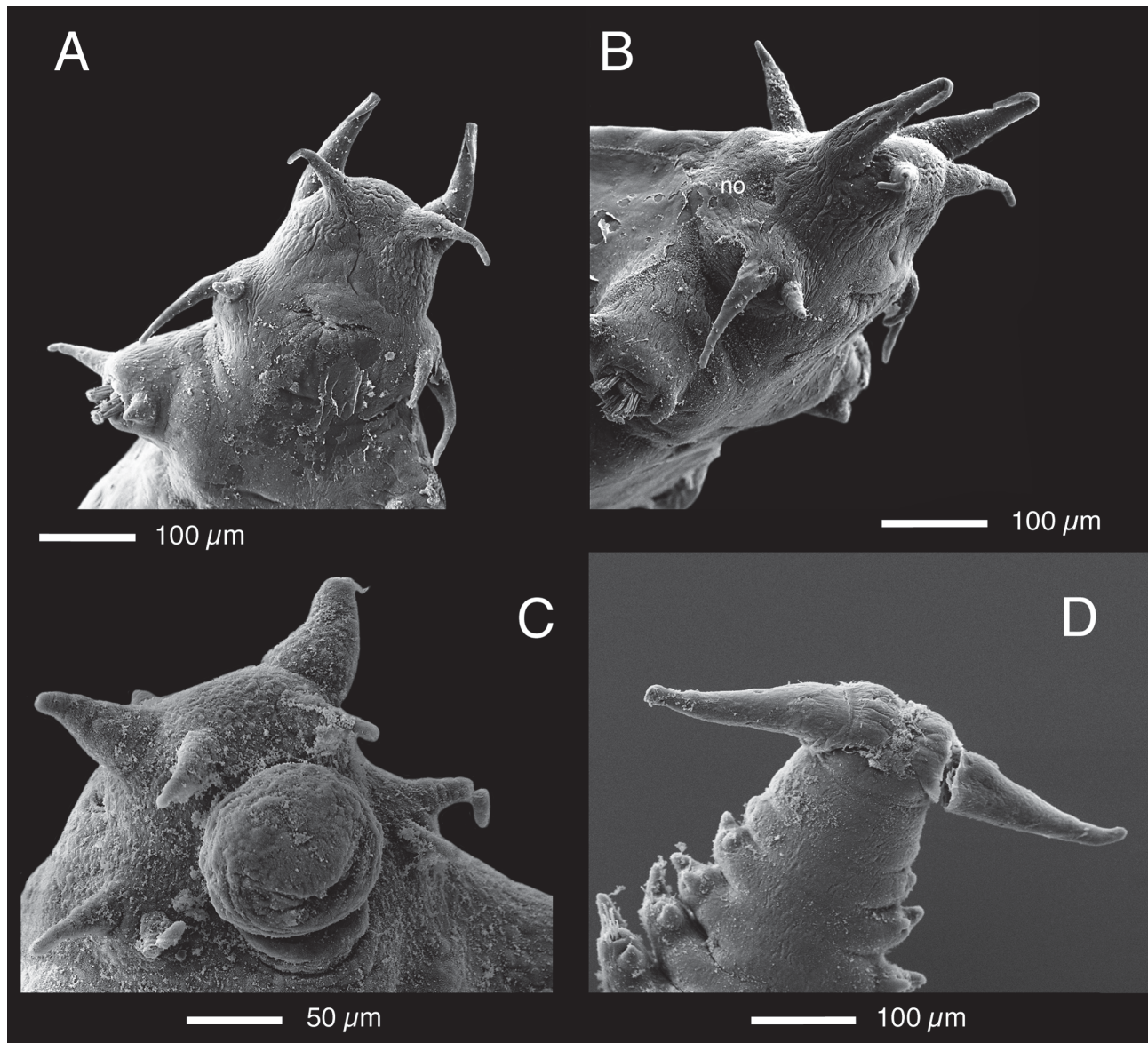


FIGURE 2. *Antonbruunia sociabilis* sp. nov. (A, B & D, NMW-Z.2012.074.10; C, NMW-Z.2012.074.12). A, Anterior region, ventral view; B, Anterior region, antero-lateral view (no = nuchal organ); C, Anterior region, ventral view, showing protruded proboscis; D, Pygidium, ventral view.

Parapodia simple, short cuff-like, lacking any marked pre- or post-chaetal structures. Dorsal cirri above or slightly dorso-posterior to parapodia; cirri somewhat conical with broad bases and tapering cirriform tips of variable extension (Figs 1A–C & 3A–B). Ventral cirri positioned below or slightly posterior to neurochaetae. Ventral cirri of anterior and posterior parapodia short, conical with bluntly pointed tips (Fig. 3A,D). Those of median body larger, more rounded, or rounded with tiny sharply pointed tips (Figs 1C, 3C). Anterior parapodia supported by two parallel, robust and distally pointed neuroaciculae; increasing to three aciculae by about chaetiger

6 or 7. Single notoaciculæ with sharply tapering tips arise separate from neuroaciculæ (Fig. 1C). Each notoacicula directed forward into anterior part of parapod, tip ending anterior to and level with uppermost neurochaetae, well in advance of dorsal cirrus (Fig. 1B,C); in slide preparations notoacicula may seem closer to dorsal cirrus than reality. Neurochaetae numerous in anterior and mid body region, disposed in two bundles; superior bundle compact, inferior bundle broader with chaetae extending more dorsally around acicular lobe on posterior side (Figs 1B, 3E). Number of chaetae similar in each bundle, usually slightly more in inferior bundle; two bundles together totalling about 30–35 chaetae in anterior parapodia, but increasing to 45–50 in parapodia of largest segments. Separation between superior and inferior bundles disappears in posterior parapodia, and number of chaetae decreases to less than five or six on posteriormost segments. Posteriormost 4–6 segments of smallest specimens have rudimentary parapodia and no chaetae. Neurochaetae slender, shafts smooth, each with strongly bent distal tooth and longer slender and delicate secondary spine, space between tooth and spine angular (Figs 1E, 3F); primary teeth all pointing dorsally (Fig. 3E).

Reproduction. Largest specimens, longer than about 15 mm clearly female with small (ca. 25 µm diameter) oocytes in parapodia and loose in coelom (Fig. 1F). Several specimens between approximately 11 and 15 mm appear to be immature females. Sex of smaller specimens not determined.

Colour. Preserved specimens white. Colour of live animals not known.

Methyl Green staining. Glandular staining variable, most pronounced on the larger specimens. Band of speckles occurring behind the mouth and behind the ventral tentacular cirri. Ventrally, anterior 9 or 10 chaetigers with two widely separated, and interrupted, parallel lines of stain. These lines linked in posterior part of each chaetiger by broad transverse band, producing repetitive H-pattern of stain on anterior venter. The transverse band disappears on following chaetigers, and only two broken parallel lines remain.

Other staining associated with the parapodia. Ventral staining patch present at base, as vertical line on body just anterior to each parapod, and as vertical patch on its anterior face. Similar, but more sparse speckled areas on posterior face of parapod. Sparse speckles of stain evident on basal part of dorsal cirri and on body dorsal to parapodia; mid-dorsal region unstained. Pygidial lobes surrounding anus stained.

Morphometrics. The morphological attributes measured (width, length, number of segments) for *A. sociabilis* sp. nov. were strongly correlated: width and length (Fig. 4A), $r = 0.957$, $p < 0.0001$, $n = 14$; segments and length (Fig. 4B), $r = 0.894$, $p < 0.0001$, $n = 17$; segments and width (not figured), $r = 0.806$, $p = 0.0002$, $n = 14$.

Etymology. The species name *sociabilis* (L.), disposed to companionship, relates to the tendency for numerous individuals of the new species to live together. Further, it does honour to Roger Bamber, the most sociable of men.

Remarks. Hartman & Boss (1965) and Quiroga & Sellanes (2009) referred to all *Antonbruunia* prostomial appendages as antennae. The two antero-ventral prostomial appendages are here considered to be palps, while the two larger antero-dorsolateral organs are the lateral antennae. This is consistent with their positions on the prostomium in literature associated with Antonbruniidae (Fauchald 1977; Pleijel & Dahlgren 1998; Pleijel 2001c; Aguado *et al.* 2013) and putative related families, Pilargidae (Pettibone 1966; Fitzhugh & Wolf 1990; Fauchald & Rouse 1997; Glasby 1993, 2000; Pleijel 2001b) and Nautiliniellidae (Pleijel 2001a; Fauchald & Rouse 1997; Aguado & Rouse 2011; Aguado *et al.* 2013). However, as Glasby (1993) pointed out, the true nature of the antero-ventral appendages in *Antonbruunia* “must remain in doubt until better material is available to conduct histological study.”

Antonbruunia sociabilis sp. nov. is morphologically most similar to *A. viridis*. Segment 1, bearing the tentacular cirri, is well-defined dorsally and laterally as a distinct ring behind the prostomium. The palps and antennae are relatively slender tapering to narrow tips, median antennae and palps of similar size. Both have slender cirriform anal cirri.

In *A. gerdesi*, the tentacular cirri are more anteriorly positioned below/lateral to the prostomium, and segment 1 is dorsally reduced and narrow. All the anterior appendages are more robust, and the median antenna is similar to the lateral antennae in size and shape. The anal cirri are very robust also. Its body form seems altogether wider (Fig. 4A), though some caution must be exercised here due to potential differences attributable to fixation treatments; *A. gerdesi* material was removed from its bivalve host and then fixed in 10% formalin prior to transfer in 70% ethanol, while *A. sociabilis* was preserved inside its host when the latter was preserved directly in 70% ethanol. *Antonbruunia viridis* was likewise removed live from its host, but fixing and preservation were not reported.

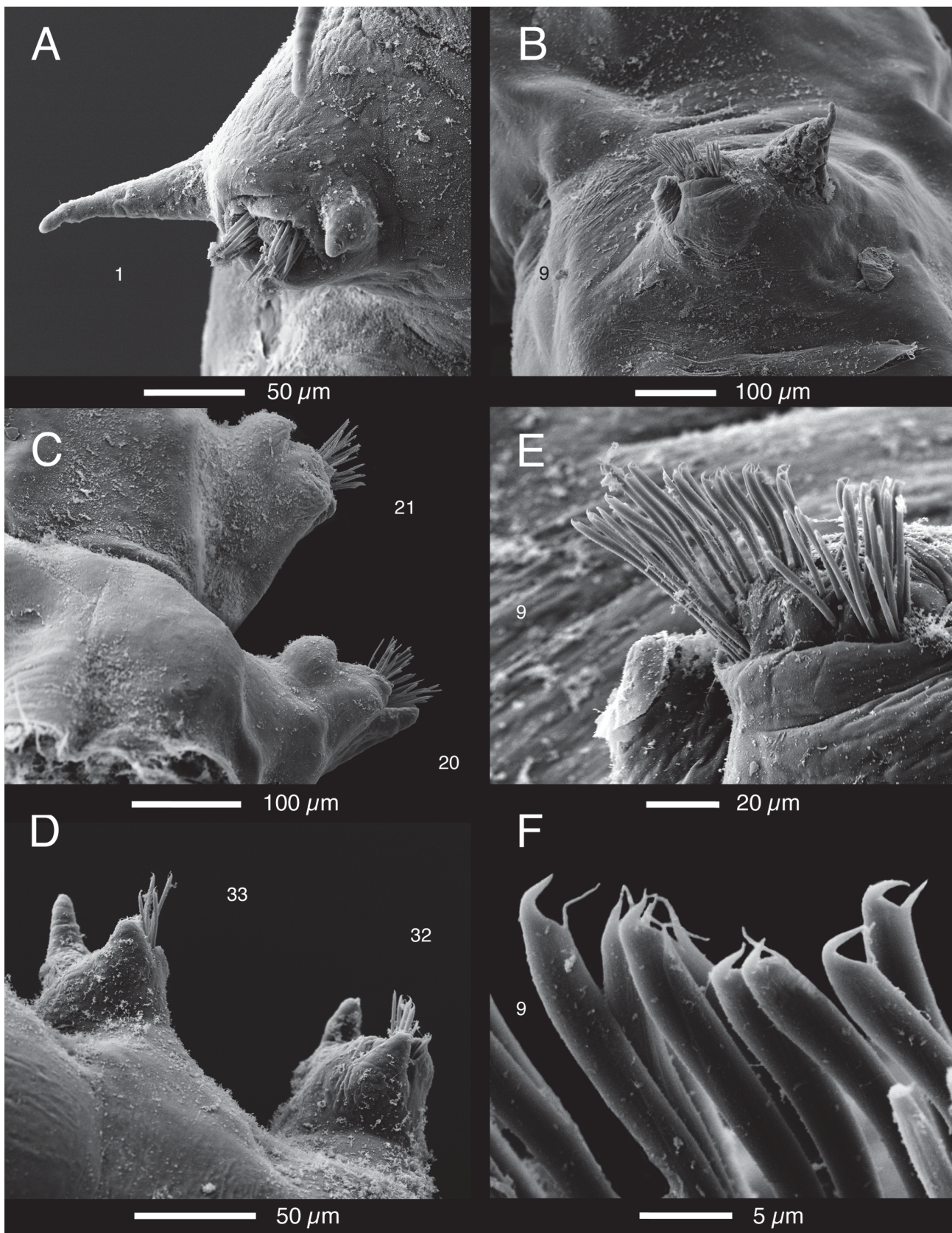


FIGURE 3. *Antonbruunia sociabilis* sp. nov. (NMW-Z.2012.074.10). A, Chaetiger 1, antero-ventral view; B, Chaetiger 9, antero-ventral view; C, Chaetigers 20 and 21, antero-ventral view; D, Chaetigers 32 and 33, postero-ventral view; E, Parapodium, ventral cirrus and chaetae, chaetiger 9, anterior view; F, Chaetae, chaetiger 9, from upper posterior part of subacicular bundle.

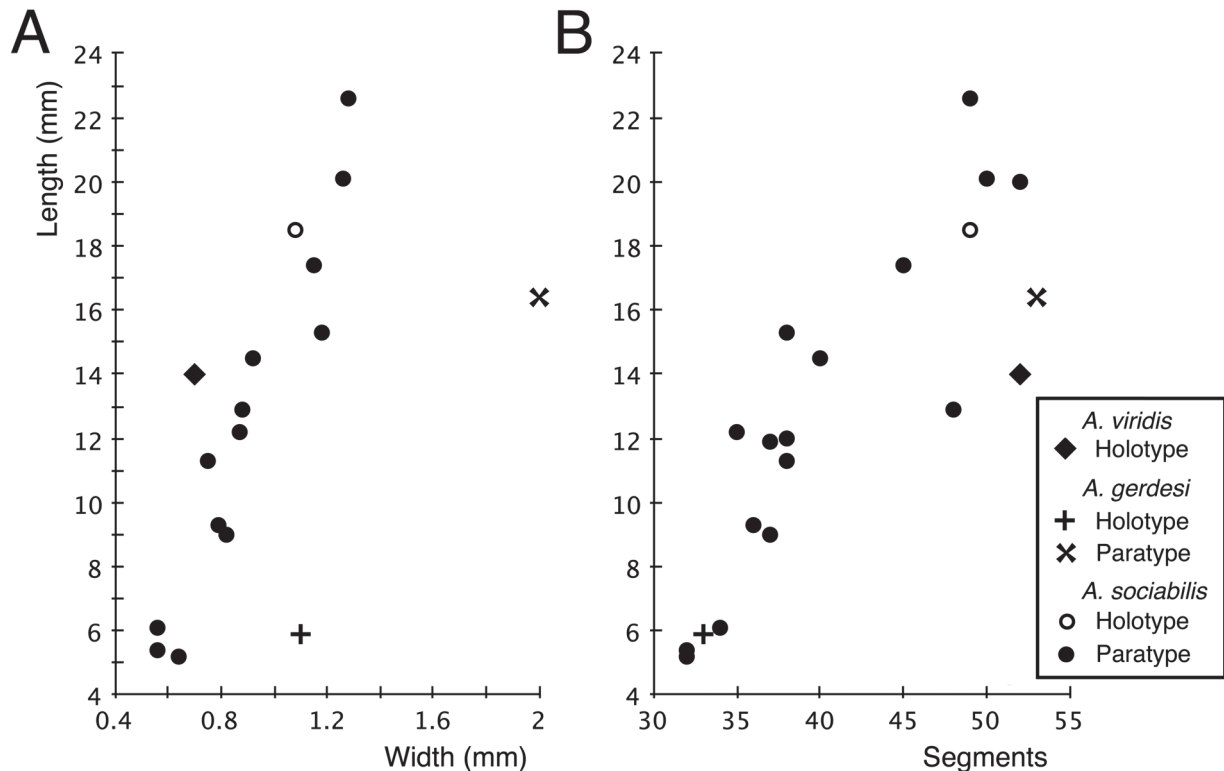


FIGURE 4. Morphometric relationships for *Antonbruunia sociabilis* sp. nov., and type material of *A. viridis* and *A. gerdesi*.

Antonbruunia gerdesi and the new species differ from *A. viridis* in having broad-based conical, rather than slender and cirriform, dorsal cirri. These two species additionally may possess more angular neurochaetae (but see Glasby 1993: fig. 3b). The new species differs from *A. viridis* in having three, rather than two (Glasby figures only one), aciculae in median chaetigers. It differs from both *A. viridis* and *A. gerdesi* in having more rounded and less protruding ventral cirri in the mid-body region; these two with short pointed ventral cirri throughout. All three species differ in the number of chaetae present. The maximum number of neurochaetae per parapodium reported was 26–30 (Hartman & Boss 1965) for *A. viridis*; Glasby (1993) depicted 35. For *A. gerdesi*, the SEM images presented by Quiroga & Sellanes (2009) revealed at least 70 neurochaetae. *Antonbruunia sociabilis* is intermediate between these two, having up to 50 neurochaetae.

The three species are widely separated geographically, occur at different depths and have different host bivalves: *Antonbruunia viridis* (Western Indian Ocean, 68–82 m, with *Lucina fosteri*), *A. gerdesi* (Southeastern Pacific Ocean, 795–846 m, with *Calymptogena gallardoi*), and *A. sociabilis* (Northeastern Atlantic, 1187–1200 m, with *Thyasira scotiae*). The first was reported to occur in male-female pairs in 80% of more than 100 *L. fosteri* animals, one bivalve hosted three individuals. The Chilean species was less prevalent with only four of 35 *C. gallardoi* animals hosting a single individual, although three occurred in another. *Calymptogena gallardoi* was unusual in additionally hosting the ‘nautiliniellid’ (now part of Calamyzinae in Chrysopetalidae, see Aguado *et al.* 2013) *Shinkai robusta* Quiroga & Sellanes, 2009 (single individuals in two of 35 bivalves), but there was no co-occurrence with *A. gerdesi*. *Antonbruunia sociabilis* is remarkable in having up to nine individuals inhabiting *T. scotiae* (maximum recorded size 19.8x19.6x12.56 mm). It was not obvious whether the three smallest specimens of *A. sociabilis* were male or simply juvenile. The presence of 4–6 small posterior segments with rudimentary parapodia may be indicative of the latter.

It is unusual to have many symbionts living in one bivalve host. In the Thyasiridae, Blake (1990) found only single examples of the calamyzin *Petrecca thyasira* inhabiting *Thyasira insignis* (Verrill & Bush, 1898) from the deep Northwestern Atlantic (3720 m). Miura & Hashimoto (1996) similarly found a single individual of *Thyasiridicola branchiatus* in *Conchocele disjuncta* Gabb, 1866 from Hatsushima cold seep, Japan (1160 m).

However, Aguado & Rouse (2011) recorded up to 25 individuals of *Laubierus alvini* within the larger (6.0–10.4 cm) Mytilidae *Bathymodiolus* sp. from methane seeps off Costa Rica, Eastern Pacific Ocean (1000–1800 m).

Phylogenetic Results

The combined data set of 16S rDNA, and 18S rDNA, excluding the alignment-ambiguous sites, consists of 2250 characters (divided on the genetic markers as 1783, and 467 characters) of which 744 are parsimony-informative (divided on the genetic markers as 274, and 470 characters), and 285 are variable but not parsimony-informative (divided on the genetic markers as 245, and 40 characters). The majority rule topologies from BA and ML of the combined data set are congruent, although the topology from BA is more resolved (Fig. 5), as is also the case for the analysis on the separate data sets (not shown). The result that *Antonbruunia sociabilis* sp. nov. is sister taxa to the two included pilargids is only supported in the BA analyses, and support for this could not be found in the less resolved ML analyses. The result is also only found in the combined data set. In the nuclear data set the position of *A. sociabilis* is more unresolved but still grouped together with the representatives of Pilargidae, Aphroditiformia, Chrysopetalidae, Nereididae, Syllidae, and Sphaerodoridae as in the combined data set. In the mitochondrial data set *A. sociabilis* is nested within Pilargidae, and it is found as sister taxon to *Sigambra* sp. (posterior probability = 0.95) with *Ancistrosyllis* sp. as the next consecutive sister (posterior probability = 0.98).

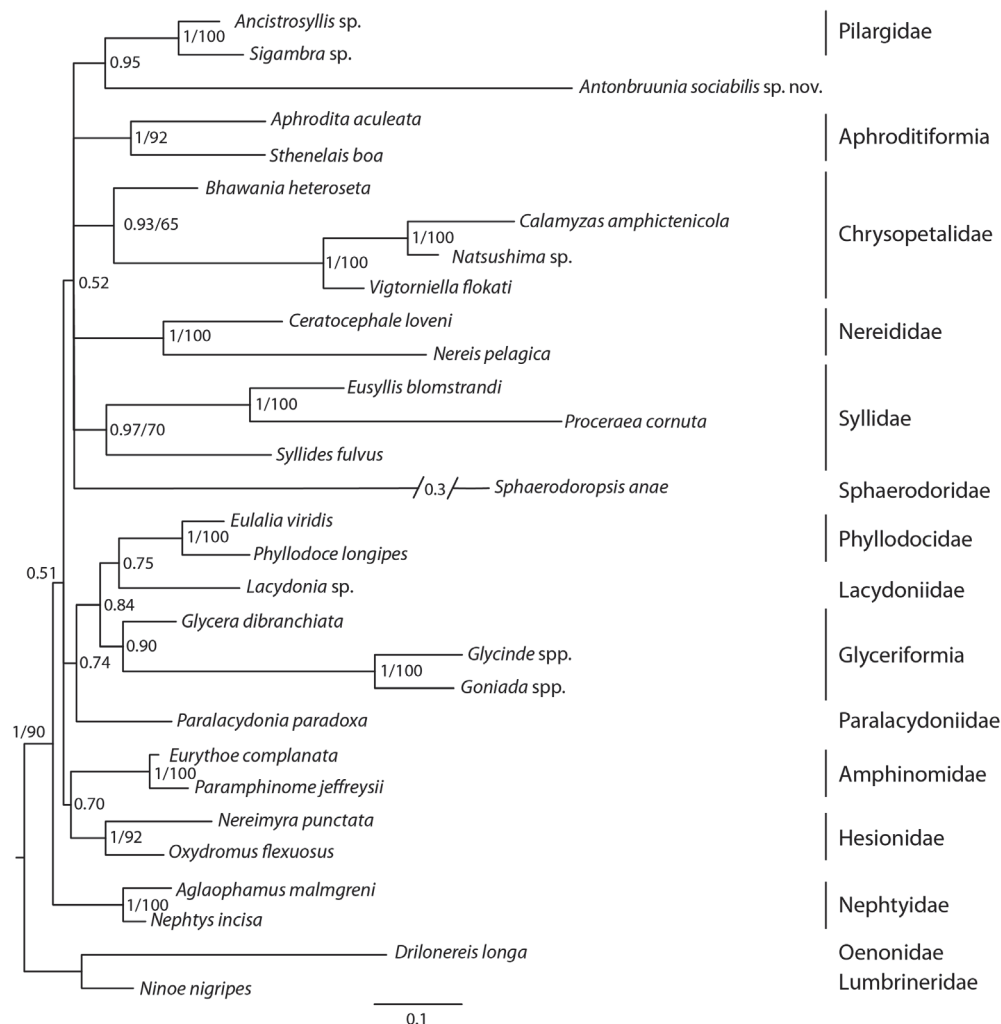


FIGURE 5. Phylogenetic tree from the combined Bayesian analysis together with support values from the maximum likelihood analysis. First node values represent posterior probabilities from the Bayesian analysis, and second bootstrap support from the maximum likelihood analysis. *Antonbruunia sociabilis* voucher (NMW-Z.2012.074.13).

Discussion

The systematic placement of *Antonbruunia* has proven problematic. Hartman & Boss (1965) noted that it shared some similarities with the Pilargidae, and several subsequent authors, placed the genus within this family (Salazar-Vallejo 1986; Salazar-Vallejo & Orensanz 1991; Glasby 1993, 2000). Fauchald (1977) however, believed it separate and erected the Antonbruunidae. Later, Miura & Laubier (1990) acknowledged resemblances between *Antonbruunia* and *Nautiliniella* (Nautiliniellidae) but, after a re-examination of *A. viridis* paratypes, concluded that the two families were morphologically distinct and that the Nautiliniellidae was “near the family Pilargidae.” Pleijel & Dahlgren (1998) found the position of *Antonbruunia* to be variable in their cladistics analyses and acknowledged that relationships between Antonbruunidae, Nautiliniellidae and Pilargidae merited further investigation. Uncertainties surrounding *Antonbruunia* persist to the present day (e.g. Martin & Britayev 1998; Aguado & Rouse 2011), although the Nautiliniellidae are now regarded as part of the subfamily Calamyzinae within the Chrysopetalidae (Aguado *et al.* 2013). The discovery of *A. sociabilis* enables us to re-assess the status of the Antonbruunidae from a morphological and molecular perspective.

Paired segmental gut caecae (diverticula) are known in a number of polychaete families; for example, some Aphroditidae (Fauvel 1923: fig. 7b; Fordham 1925; Rouse & Pleijel 2001), Spintheridae (von Graaff 1888; Manton 1967) and Syllidae (Saint-Joseph 1887; Fauvel 1923: 270–271). Müller (1858) noted lateral extensions of the gut when describing the pilargid *Sigambra grubii* (see also Pettibone 1966; Salazar-Vallejo 1990), but otherwise these structures have not been documented in other Pilargidae. Gut caecae have not been noted previously in Chrysopetalidae (including former nautiliniellids), but do occur in *Calamyzas amphictenicola* Arwidsson, 1932 ectoparasitic on the ampharetid *Amphicteis gunneri* M. Sars, 1835. The significance of their presence in *Antonbruunia sociabilis* is unknown; gut caeca in other polychaetes are presumed to increase the absorption area of the intestine.

Antonbruunia and bivalve-inhabiting Calamyzinae appear to have very similar morphologies. Their head regions and sensory appendages (though lacking the median antenna in calamyzins) and ventral mouth are nearly identical. They both have neuropodia with acicula(e), neurochaetae and ventral cirri, lack notochaetae and have dorsal cirri. However, closer inspection reveals many differences:

Segment 1 differs between the two. In *Antonbruunia* segment 1 is delineated from the prostomium, always has two pairs of subequal tentacular cirri and lacks chaetae. In calamyzins segment 1 is generally fused with the prostomium and a somewhat ventrally displaced pair of parapodia (with chaetae) is often present. This has led to differing interpretations of the anterior appendages and Aguado & Rouse (2011) made the case for the ventral ones being palps, rather than ventral cirri of segment 1. Segment 1 often bears dorsal (or tentacular) cirri also, but arrangements differ considerably, and cirri, parapodia and chaetae may be variously lacking (e.g. as in *Mytilidiphila* Miura & Hashimoto, 1993). All are lacking in *Iheyomytilidicola* Miura & Hashimoto, 1996.

Multiple neuropodial and single notopodial aciculae are present in *Antonbruunia*, the latter gently curved and projecting forward away from the dorsal cirrus, terminating anterior to the superior neurochaetae. Thus the parapodia appear superficially uniramous. In calamyzins, single robust neuropodial aciculae occur. Notoaciculae may be slender, robust or absent. When present they are single and support a distinct notopodial lobe, of various species-specific length, and terminating in a dorsal cirrus (Miura & Hashimoto 1996: fig. 1b). Dorsal cirri are not always delineated from notopodia (e.g. Miura & Hashimoto 1996: fig. 7d), and may sometimes be penetrated by the tip of the notoaicula (Miura & Hashimoto 1993: fig. 1e,f).

In *Antonbruunia* the neurochaetae are arranged in two subequal bundles, a compact supraacicular and a broader subacicular group. In calamyzins, the neurochaetae are subacicular as in other chrysopetalids (Watson Russell 2000); some chrysopetalids may have a simple supraacicular chaeta (e.g. Watson Russell 1998: fig. 3a).

Neurochaetae in *Antonbruunia* are simple, with smooth shafts and a distal hook, accompanied by a slender and delicate secondary spine. Such chaetae are present in some Pilargidae (*Pilargis* Saint-Joseph, 1899, and *Ancistrosyllis* McIntosh, 1879; e.g. Hartman 1960: pl. 7, fig. 6) and Polynoidae. Bivalve-dwelling calamyzins have neurochaetae that are superficially simple; however, there is some evidence that they may be derived from the fusion of shafts and blades of compound chaetae (Aguado & Rouse 2011; Aguado *et al.* 2013). They are often unidentate, sometimes bidentate (e.g. in *Natsushima bifurcata* Miura & Laubier, 1990; *Iheyomytilidicola lauensis* Aguado & Rouse, 2011) or trifurcate (e.g. *Vesicomycicola trifurcatus* Dreyer *et al.*, 2004). Several different forms may occur in the same parapodium. Compound neurochaetae occur in other chrysopetalids, as well as the calamyzin genera *Calamyzas* and *Vigtorniella*.

Antonbruunia has a pair of ventrolateral anal cirri, whereas these are lacking in bivalve inhabiting calamyzins. Two anal cirri are present in *Vigtorniella* and Chrysopetalinae.

By contrast the similarities between *Antonbruunia* and Pilargidae, particularly *Pilargis* and *Ancistrostylis*, are much more numerous. *Antonbruunia* neurochaetae strongly resemble those of these genera; compound chaetae are absent in Pilargidae. Notoaciculae are likewise single, curved, and positioned anterior or antero-dorsal to the dorsal cirri (e.g. Katzmann *et al.* 1974: figs 6a, 7a) – albeit somewhat closer to the cirri than in *Antonbruunia* – and there are no notopodial lobes. The neurochaetae in Pilargidae are both sub- and supra-acicular (Pettibone 1966; Katzmann *et al.* 1975; Darbyshire & Mackie 2003) and some genera, including *Pilargis*, lack notochaetae. The pygidium bears a pair of anal cirri. Anteriorly, most pilargids also have a pair of tentacular cirri on a defined segment 1, as well as a pair of palps and a pair of lateral antennae. A dorsal median antenna may be present or absent. *Antonbruunia* differs in its narrower and more ventrally (rather than antero-ventrally) located mouth, and smaller palps. Pilargid palps are sometimes large with small palpostyles, and can exhibit various degrees of fusion in different genera. However, as *Antonbruunia* resembles ‘nautiliniell’ calamyzins in these respects, these features may be convergent adaptations for a symbiotic existence within bivalves.

The molecular results clearly indicate that a close relationship to Pilargidae (represented by *Ancistrostylis* sp. and *Sigambra* sp.) is more likely than an affinity to Calamyzinae (represented by *Calamyzas amphictenicola*, *Natushima* sp., and *Vigtorniella* sp.). However as support for *Antonbruunia* as sister taxa to Pilargidae only is found in the Bayesian analyses of the combined data set, the matter needs further investigations before it can be settled. The mitochondrial 16S rDNA on its own places *Antonbruunia* within Pilargidae, while the nuclear 18S rDNA was more inconclusive to the position of *Antonbruunia*. A broader taxon sampling of representatives from Pilargidae as well as more genetic markers may help to resolve the question. We did put considerable effort to sequence a number of additional genetic markers (e.g. 28SrDNA, COI, and Histone H3) but failed, probably due to poorly fitting primers.

Polychaete-bivalve relationships

The genus *Antonbruunia* has only been found as an inhabitant of bivalve mantle cavities. The bivalves belong to three distinct families, Thyasiridae, Lucinidae and Vesicomidae, taken from three oceans and from shelf to abyssal depths. The only common feature of the bivalves is that they are chemosymbiotic. A similar bias towards chemosymbiotic bivalves is also shown by the Nautiliniellidae having been found in the Solemyidae, Vesicomidae, Thyasiridae, and Bathymodiolinae, but not in suspension or deposit feeding taxa. The Bathymodiolinae are also host to a number of Polynoidae. The presence of inquiline polychaetes inhabiting the mantle cavity of suspension feeding or deposit feeding bivalves is rare; Martin & Britayev (1998) recording only four out of the 40 species listed as associated with such bivalves.

The hesionid *Parasyllidea humesi* Pettibone, 1961 is monoxenous with deposit feeding tellinoid bivalves and appears to affect the metabolism of the bivalve (Martin *et al.* 2012) while the polynoid *Branchipolynoe seepensis* Pettibone, 1984 causes damage to the organs of the mantle cavity (Britayev *et al.* 2007). While vent and seep chemosymbiotic bivalves are prone to infestation, *Antonbruunia* has also been found in a tropical shelf species of Lucinidae further suggesting that the key factor is the bacterial symbiosis rather than the vent and seep environment. Even so infestation is rare, occurring in only three of the 120 species of Thyasiridae and in one of the 500 species of Lucinidae despite the recent exhaustive review of this latter family (Taylor & Glover 2006; John Taylor, pers. comm.). The relatively more frequent infestation of *Bathymodiolus* may be due to the epibyssate mode of life and generally unfused mantle edges that would allow access to the mantle cavity. Thyasirids and lucinids are deep burrowing forms, access to the mantle cavity is only by the anterior inhalant tube. Infestation and transfer between bivalves must be more fortuitous than in the dense beds of surface living bathymodioline mussels. The nature of the symbiotic association between *A. sociabilis* and *Thyasira scotiae* is not known, as is often the case with other polychaetes and their hosts (Martin & Britayev 1998). No damage to the bivalve tissues has been reported for any of the *Antonbruunia* species and, given the anatomy of the mouth and pharynx, it is difficult to see how these polychaetes could be feeding on bivalve tissues. Additionally, the frequent presence of many *A. sociabilis* individuals living within single *Thyasira scotiae* would not seem indicative of a parasitic relationship. Hartman & Boss (1965) indicated that the head of *A. viridis* “was directed towards the siphons”. Actually their

illustration shows the worm heads facing the inhalant aperture, and the larger female wrapped around the foot. No pattern of orientation was observed in *A. sociabilis* with the worms entangled in the arborescent lateral body pouches (Oliver & Drewery 2014: fig. 9a).

The calamyzins *Petrecca thyasira* Blake, 1990, *Natsushima graciliceps* Miura & Hashimoto, 1996, *Thyasiridicola branchiatus* Miura & Hashimoto, 1996, *Iheyomytilidicola lauensis* Aguado & Rouse, 2011, *Laubierus alvini* Aguado & Rouse, 2011 and *Natsushima sashai* Aguado & Rouse, 2011 have been reported as living between bivalve gill filaments. Indeed, when describing *Mytilidiphila enseiensis* and *M. okinawaensis*, Miura & Hashimoto (1993) stated that “Most nautiliniellid worms attached so stiffly to or penetrated so deeply into the gill filaments of the host mussels that we had to dissect the host tissue for collection.” *Nautiliniella calyptogenicola* (Miura & Laubier, 1989) (Sato-Okoshi 2003), *Shinkai longipedata* Miura & Ohta, 1991 (Aguado & Rouse 2011) and *S. fontefridae* Aguado & Rouse, 2011 have been found between the gill lamellae and the foot of vesicomid *Calyptogena* species. Most other former nautiliniellids were simply recorded as occurring in the mantle cavities of their hosts (Miura & Laubier 1990; Miura & Hashimoto 1996; Dreyer *et al.* 2004; Quiroga & Sellanes 2009), but three species (Blake 1993) were collected free in sievings and could not be linked to any particular bivalves.

The possible food sources for a non-parasitic polychaete could be the feeding strings of particles collected by the bivalve on the gills and palps or the pseudofaeces aggregated along the inner mantle edge. Such sources are however present in all bivalves, not only chemosymbiotic taxa, so it is probable that these polychaetes are in some ways dependent on the bacterial communities associated with the bivalves. The bacteria are variously associated with the gills; e.g. intercellular or extracellular in deep-sea bathymodiolins, intracellular in Vesicomidae, inside bacteriocytes in Solemyidae, and associated with epithelial vacuoles in Lucinidae (Duperron *et al.* 2013). For the Thyasiridae the situation is diverse, ranging from species lacking chemosynthetic bacterial symbionts to those with “very dense bacterial populations in the lateral zone of gill filaments”, the bacteria packed into large bacteriocytes in the gill epithelium. The bacteria in chemosynthetic host bivalves are mainly sulphide and/or methane oxidisers (Duperron *et al.* 2013: table 1). There is no evidence as yet that the polychaetes themselves are chemosymbiotic. It is of interest that one of the three species of *Vigtorniella* Kiseleva, 1996 (Calamyzinae), *V. ardebilia* Wiklund *et al.*, 2009, has been observed feeding on giant filamentous bacterial mats associated with whale bones and also likely feeds on bacterial mats under fish cages. Another, *V. zaikai* (Kiseleva, 1992) inhabits sulphide-rich sediments.

Chemosynthetic symbiotic bacteria are prevalent in the bivalve hosts of Calamyzinae and Antonbruunidae. An ultrastructure examination would reveal the makeup of the gut contents or whether any of the tissues supported symbiotic bacteria. Unfortunately the *A. sociabilis* material was inappropriately fixed for transmission electron microscopy. Wider morphological examinations may reveal whether ‘commensal’ Antonbruunidae and Calamyzinae are derived from free-living bacterial feeding forms.

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